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Award Number: W81XWH-14-1-0006

TITLE:
Preventing Ototoxic Synergy Of Prior Noise Trauma During Aminoglycoside Therapy

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Portland, OR 97239

REPORT DATE: December 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2015	Annual Report	1 Dec 2014 - 30 Nov 2015

4. TITLE AND SUBTITLE

Preventing Ototoxic Synergy Of Prior Noise Trauma During Aminoglycoside Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0006

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status. The combination of both prior noise trauma and aminoglycoside treatment can degrade auditory function greater than simple summation of the two insults. We have found that prior sound exposure enhances cochlear uptake of aminoglycosides, providing a mechanistic basis for the observed ototoxic synergy due to noise trauma and subsequent aminoglycoside treatment.

15. SUBJECT TERMS
Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, ototoxicity, auditory function, hearing loss

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION

Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status. The combination of both prior noise trauma and aminoglycoside treatment can degrade auditory function greater than simple summation of the two insults. We have found that prior sound exposure enhances cochlear uptake of aminoglycosides, providing a mechanistic basis for the observed ototoxic synergy due to noise trauma and subsequent aminoglycoside treatment.

In the mammalian inner ear – the cochlea, the auditory sensory cells, particularly outer hair cells (OHCs), are more susceptible to aminoglycoside-induced cytotoxicity than other cochlear cells, particularly at the base of the cochlea most sensitive to higher frequency sound. Once these OHCs are lost, these sensory cells cannot be endogenously regenerated, leading to life-long hearing loss and deafness. Thus, extensive efforts are underway to ameliorate and prevent aminoglycoside-induced hair cell death. Under normal physiological condition, aminoglycosides can rapidly cross the blood-labyrinth barrier (BLB) into the cochlear tissues and fluids and enter OHCs through a number of conduits. The best-characterized conduit is permeation through the mechanoelectrical transduction (MET) channel. The MET channel is mechanically-gated by the extracellular, heterodimeric tip links between two stereocilia. Other mechanisms by which aminoglycosides can enter hair cells include endocytosis, and/or other aminoglycoside cation channels (*e.g.* TRP channels) expressed by hair cells besides the MET channel, such as TRPV4 on the apical membranes, or TRPA1 on the basolateral membranes, of OHCs.

The ultimate goal of this research is to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, including combatants and associated casualties to pre-injury effectiveness. In this project, we hypothesize that prior noise trauma induces synergistic ototoxicity with systemically-administered aminoglycosides by potentiating cochlear uptake of the drug. We also hypothesize that specific aminoglycoside-permeant cation channels directly facilitate noise trauma-enhanced uptake of aminoglycosides in the cochlea.

KEYWORDS

Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, ototoxicity, auditory function, hearing loss

ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1: Determine the acoustic parameters that induce noise-enhanced aminoglycoside uptake in auditory sensory hair cells.

This is completed by the end of Year one.

Aim 2: Determine if prior noise trauma modifies intra-cochlear trafficking of aminoglycosides.

Aim 2a: Use cochlear perfusion techniques to determine the contribution of endolymph or perilymph trafficking of aminoglycosides to hair cells with prior noise exposure. GTTR will be administrated either systemically or by scala tympani infusion to the animal.

This is completed by the third Quarter of Year two.

Aim 3: Determine if aminoglycoside-permeant channels on the hair cell apical membrane contribute to aminoglycoside uptake by cochlear hair cells.

Aim 3a: Determine if prior noise trauma enhances drug uptake in hair cells, by using mouse models with MET apparatus defects, including *Pcdh15*$^{3J/3J}$ (Ames waltzer) mice, *Myo7a*$^{8J/8J}$ (Shaker 1) mice; and *TrpV4*$^{-/-}$ and *P2X2*$^{-/-}$ mice with channelopathies, compared to heterozygous littermates.

This is ongoing during the third Quarter of Year two.

Aim 4: Determine if TRP channels on the basolateral membrane of cochlear hair cells also contribute to aminoglycoside uptake.

What was accomplished under these goals?

1) Major activities

a) Conducted experiments to characterize strial capillary dilation in mouse models of sound-enhanced intro-cochlear aminoglycoside trafficking.

b) Accumulated adequate number of *TrpV1* mice (Cat#3770, Jackson Laboratories), and characterized the mutant mice by ABR testing.

c) Accumulated adequate number of *Myo7a* mice, and characterized the hair cell survival in adult mutant mice.

d) Studied the pattern of aminoglycoside uptake by cochlear sensory hair cells in neonatal and adult mice.

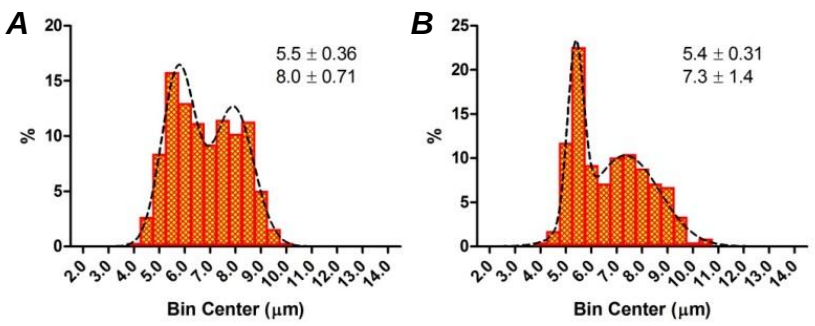


Figure 1*. Sound-induced capillary vasodilation and the effect of cardiac perfusion with PFA. Vasodilation of strial capillaries was evident from the basal region of sound-treated cochleae, showing a characteristic bi-modal distribution (**A**; 718 total measures). This distribution was not significantly different from strial capillary diameters from mice with cardiac perfusion fixation after 96 dB SPL, WBN sound exposure (**B**; p=0.29, Mann Whitney test). The value and standard deviation (S.D.) of the mean in μm are provided in each panel. Curve fitting in bi-model Gaussian distribution is depicted by a dotted line.*

2) Specific objectives

a) Establish image processing techniques for diameter measurement of strial capillaries.

b) Measure capillary diameters in control stria vascularis, and in sound-treated strial vascularis.

c) Examine the effect of cardiac perfusion on sound-induced vasodilation in strial capillaries.

d) Establish and expand *TrpV1* mouse cohort.

e) Immuno-labeled cochlear and kidney tissue from both heterozygous and mutant *TrpV1* mice, using TRPV1 antibodies from two different manufactures.

f) Characterized the effect of *TrpV1* deletion on auditory sensitivity at multiple postnatal ages, by measuring auditory brainstem response (ABR).

g) Establish and expand *Myo7a* mouse cohort.

h) Quantified outer (OHC) and inner hair cell (IHC) survival in young adult mutant mice.

i) Systemically administered fluorescently tagged gentamicin (GTTR) in mutant neonatal mice, or in adult mice.

j) Characterized the effect of *Myo7a* mutation on the uptake of GTTR, by examining OHC survival in the organ of Corti.

3) Significant results or key outcomes

a) The diameters from individual stack/site from individual control animals exhibited a Gaussian/normal distribution. The mean diameter varied among different cochlear locations. Strial capillaries from the cochlear base present larger diameters than the cochlear apex (n.s., p=0.25, unpaired t test with Welch's correction), whilst strial

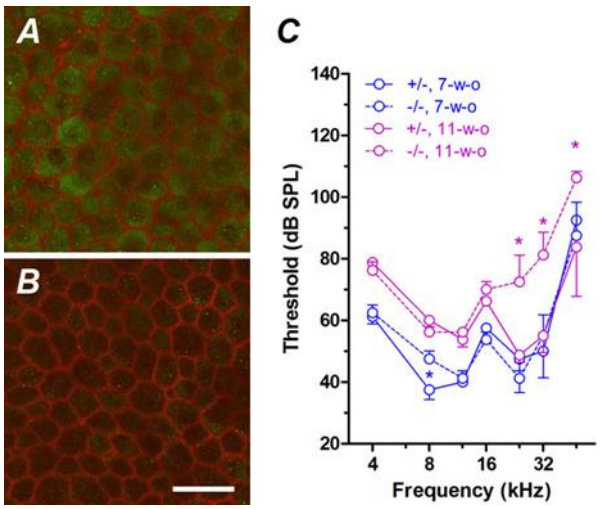


Figure 2*. Protein expression and hearing sensitivity in TrpV1 mice.* ***A****: TRPV1 was immuno-localized in marginal cells of the stria vascularis in heterozygous mice (control).* ***B****: Negligible immuno-positive TRPV1 signal was observed in mutant mice. Scale bar = 20 µm. C: Baseline hearing sensitivity of mutant mice was comparable to littermate controls at 7 weeks of age. High frequency hearing loss was evident in older 11 week old mutant mice (* p<0.05).*

capillaries from the mid-cochlear coils present significantly smaller diameters ($p<0.0001$, compared to base; $p<0.0009$, compared to apex; unpaired t test with Welch's correction).

b) After wide band noise (WBN) exposure at 96 dB SPL of 18 hours (6 hours/day for 3 days), a fraction of the strial capillaries retained their baseline diameter, while others dilated, forming a bi-modal distribution. Cardiac perfusion as part of paraformaldehyde (PFA) fixation did not affected capillary vasodilation (Fig. 1).

c) Immuno-labeled TRPV1 protein was overt throughout the stria vascularis of the cochlea (Fig. 2A; exemplified in marginal cells). Cytoplasmic punctate labeling was observed in the interstrial layer and basal cell layer. In the kidney, punctate labeling was also observed in the epithelial cells of convoluted tubules near the brush border (data not shown). Negligible immune-labeling was observed in the cochlea (Fig. 2B) or in the kidney. Superior microscopic result was achieved by using TRPV1 antibody from Alomone Labs.

d) No hearing deficit was previously reported in *TrpV1* mice. Our initial data confirmed the auditory sensitivity in mutant mice was comparable to their littermates at 7 weeks of age (Fig. 2C; blue lines). Interestingly, as mice age, mutant mice exhibited evident high frequency loss, examined at the age of 11 weeks (Fig. 1C; magenta lines) and 18 weeks. Thirty-to-40 dB elevation was seen between 24-48 kHz.

e) Unlike wildtype or heterozygous littermate mice, the mutant *Myo7a*$^{8J/8J}$ mice are known of severe hearing loss. Indeed, here we observed massive hair cell loss in 5-week-old young adult mutant mice (Fig. 3A&B). The cell loss was more severe for OHCs compared to IHCs at the same frequency locations; the loss was also more severe at frequencies above 16 kHz, with large variation among individual animals (Fig. 3C&D). However, at frequency 12 kHz and below, substantial hair cells were surviving at this age, granting experiments to study gentamicin uptake by hair cells.

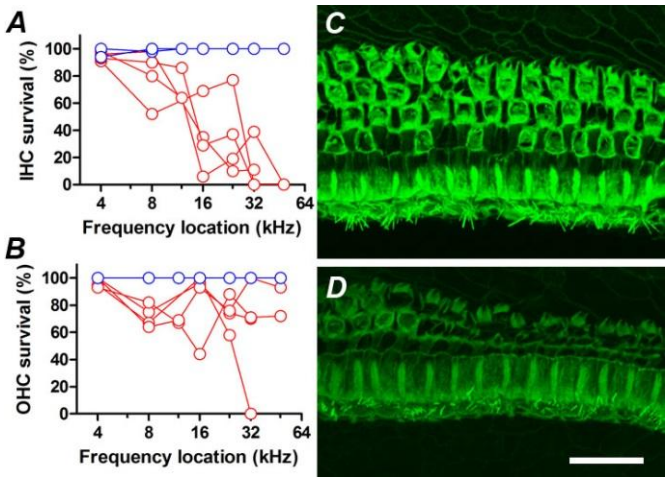


Figure 3*. Hair cell loss in adult Myo7a mutant mice.* ***A****: Massive OHC loss was observed from 4 individual 5-week-old Myo7a$^{8J/8J}$ mutant mice (red), while little or no cell loss from 2 littermate control Myo7$^{8J/+}$ heterozygous mice (blue).* ***B****: Similarly, IHC loss was observed from the same group of mutant mice (red), while no cell from control group (blue).* ***C****: With phalloidin labeling, surviving but unhealthy OHCs could be seen from some mouse at 24 kHz cochlear region. The hair bundle of IHCs was identifiable with disarrayed stereocilia.* ***D****: In other mouse, OHCs were largely gone at 24 kHz cochlear region. Scale bar = 25 μm.*

4) Other achievements:

a) We also tested the effect of shorter, more intense sound treatment (WBN; 110 dB SPL, 2 h) on strial vasodilation. The diameter measurement was performed from the mid-base region of the cochlea. A Gaussian distribution was evident from the control tissue (n=3; 367 measures). In sound-treated stria vascularis, capillaries were moderately dilated without a clear bi-modal distribution (n=4; 487 measures). However, this vasodilation was significant ($p<0.0001$, unpaired t test).

b) Completed institutional IACUC 3-year renewal that includes this TATRC/ USAMRAA award and ACURO documentation. Received protocol approval from ACURO.

c) We also examined neonatal *Myo7a$^{8J/8J}$* mutant mice, from P7-P11, and did not observe any overt hair cell loss, though hair bundle structures were abnormal. GTTR uptake could be visualized in *Myo7a$^{8J/8J}$* mutant mice. Further data and image analysis is underway.

What opportunities for training and professional development has the project provided?

This research project provided opportunities for people with interest and motivation in biomedicine research, including college students and international physicians. For instance, Stanley Feng, a junior student Oregon State University, has involved in this project through OHSU research volunteer mechanism for two years. Meifang Xiao, a visiting physician who is practicing traditional medicine in Shanghai, China, also joined the research group and had hands-on experience in cochlear dissection, and image acquisition etc. This experience will certainly provide positive impact on their career development.

How were the results disseminated to communities of interest?

Part of the content in this report has been published at the 2015 Midwinter meeting of Association for Research in Otolaryngology, Baltimore MD, as attached in the Appendix. Three peer-reviewed articles, of which two are related to this project, were published during the past report period. Documentation on the auditory sensitivity in *TrpV1* mice will be reported at the 2016 Midwinter meeting of Association for Research in Otolaryngology, San Diego CA.

What do you plan to do during the next reporting period to accomplish the goals?

The PI relocated his research program from Oregon Health & Science University to Loma Linda Healthcare System in California. The transition occurred during the 3rd quarter of Year 2 of this project. The award will be transferred to the new institute, and the process is ongoing. Upon the completion of the transfer, research activity will be resumed from where it was interrupted. Considerable effort will be applied for equipment acquisition, assembling, and calibration etc. Mouse colonies will be re-established in the new animal facility. We are aiming for a smooth research transition.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

During the first a few months of research in Year two, we continued our previous work in sound-induced vasodilation that occurs in the stria vascularis. We propose that this event underlies sound-enhanced gentamicin uptake in the strial tissue and by the hair cells. Additionally, we found vasodilation could happen in a bi-model fashion, and the observation was not affected by paraformaldehyde fixation.

Candidate aminoglycoside channels (e.g. TRPV1) and their regulating components (e.g. Myo7a as part of MET apparatus) in the inner ear, control hearing sensitivity in characteristic fashion.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Aminoglycoside antibiotics, like gentamicin and tobramycin, are clinically-essential antibiotics for treating life-threatening Gram-negative bacterial infections. They are being pervasively used, seeing wide application in outpost clinics to national army/veterans hospital. In civilian population, they are particularly used in premature babies, and for patients with cystic fibrosis, or Gram positive infections like tuberculosis and protozoal infections. Despite their wide use, broad-spectrum, bactericidal efficacy and low cost, clinical dosing with aminoglycosides is limited by the risk of acute nephrotoxicity and life-long ototoxicity, with significant ramifications for quality of life. This research is to search countermeasures to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, as well as civilians received aminoglycoside therapy with a history of (or likely ongoing) acoustic insult.

CHANGES/PROBLEMS

Project transfer, and building a new research program / laboratory are both time consuming process. Extensive efforts are expected during this process, including recruiting, heavy purchasing, and protocol preparation etc., in addition to regular academic research activities. We are determined to minimize the interruption brought by award transfer, and will resume related research activities as quickly as possible.

Hair cell morphology in *Myo7a* mutant mice appears unhealthy, suggesting abnormal cellular physiology, including altered cationic inward current. We will be extra cautious when using these hair cells as targets to investigate sound-enhanced aminoglycoside uptake. Similar caution will also be applied to *Pcdh15* mutant mice, as the project advances into corresponding phases.

PRODUCTS

Peer reviewed publication

Hongzhe Li, Allan Kachelmeier, David N. Furness, Peter S. Steyger (2015). Local mechanisms for sound-enhanced aminoglycoside entry into outer hair cells. Frontier in Neuroscience 9. doi: 10.3389/fncel.2015.00130

Jawon Koo; Lourdes Qunitanilla-Dieck; Meiyan Jiang; Jianping Liu; Zachary D. Urdang; Hongzhe Li; Peter S. Steyger (2015). Endotoxemia-mediated inflammation enhances cochlear uptake of aminoglycosides and subsequent cochleotoxicity. Sci. Trans. Med. 2015 Jul 29;7(298):298ra118. doi:10.1126/scitranslmed.aac5546

Lina Reiss, Gemaine Stark, Anh Nguyen-Huynh, Kayce Spear, Hongzheng Zhang, Chiemi Tanaka, Hongzhe Li. Morphological Correlates of Hearing Loss after Cochlear Implantation and Electro-Acoustic Stimulation. Hear. Res. doi:10.1016/j.heares.2015.06.007

Conference papers and presentations

Jianping Liu, Meiyan Jiang, Zachary Urdang, Hongzhe Li, Peter Steyger (2015), "Conditional macrophage depletion by Diphtheria Toxin affects the macrophage in the cochlear lateral wall", Midwinter Research Meeting in Otolaryngology.

Yuqin Yang, Hongzhe Li, Peter Steyger and Zhi-Gen Jiang (2015), "Interleukin-6 Hyperpolarizes Strial Capillary Endothelial Cells by Activation of Stretch-Gated Chloride Channels", Midwinter Research Meeting in Otolaryngology.

Hongzhe Li, Jianping Liu, Peter Steyger (2015), "Characterizing the strial capillary dilation in mouse models of sound enhanced intra-cochlear aminoglycoside trafficking", Midwinter Research Meeting in Otolaryngology.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Hongzhe Li, PhD
Project Role: PI
Nearest person month worked: 5.7
Contribution to Project: Dr. Li has performed work in experimental design, tissue harvest and processing, confocal imaging, image acquisition and quantification, data analysis, documents, reports and manuscript preparation.

Name: Peter Steyger, PhD
Project Role: Co-Investigator, Professor
Nearest person month worked: 0.7
Contribution to Project: Dr. Steyger has involved in experimental design, animal protocol compliance and manuscript preparation.

Name: Anastasiya Johnson, MS
Project Role: Research Associate
Nearest person month worked: 4.0
Contribution to Project: Ms. Johnson has performed work in ABR recordings and part of image acquisition.

Name: Allan Kachelmeier, MS
Project Role: Research Assistant
Nearest person month worked: 0.7
Contribution to Project: Mr. Kachelmeier has involved in instrument maintenance and document proofreading.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIREMENTS

Nothing to report.

APPENDICES

Poster presentation at 2015 Midwinter meeting of Association for Research in Otolaryngology, Baltimore MD.

Characterizing strial capillary dilation in mouse models of sound-enhanced intra-cochlear aminoglycoside trafficking

Hongzhe Li, PhD[1], Jianping Liu, MD, PhD[1,2] and Peter S Steyger, PhD[1]
[1]Oregon Hearing Research Center, Oregon Health & Science University, Portland, OR 97239, USA
[2]Department of Otology and Skull Base Surgery, Eye Ear Nose and Throat Hospital, Fudan University, Shanghai 200031, China

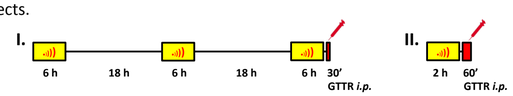
Introduction

Acoustic overstimulation potentiates the intra-cochlear trafficking of aminoglycoside antibiotics, serving as a potential mechanism of ototoxic synergy between sound and aminoglycosides, such as gentamicin [1,2]. Along with this potentiation, we observed dilated strial capillaries in fixed tissues [1]. Post-mortem analysis of capillary diameters has been questioned, as fixation may alter capillary tension and fail to faithfully preserve the anatomical diameter of capillaries and their endothelial structure. Using confocal images, we systematically quantified the diameter of strial capillaries from sound-treated and control mice to assess if meaningful physiological correlatives can be derived from such measurement and comparison.

Background: Aminoglycoside antibiotics are most frequently prescribed for prophylaxis or for treating bacterial sepsis and tuberculosis. Despite over sixty years of clinical use, it remains poorly understood how these drugs traffic from the vasculature into sensory hair cells in various clinical situations. Using cochlear perfusion techniques *in vivo*, we recently demonstrated that systemically-administered aminoglycosides traffic across blood-labyrinth barrier (BLB), and predominantly enter hair cells *via* an endolymph trafficking route [1], prior to exerting their cochleotoxicity. Additionally, hair cell uptake of aminoglycosides is increased by prior sound exposure [2], potentially correlating with pre-clinical [3-7] and clinical [8,9] observations of ototoxic synergy between loud sound exposure and aminoglycosides.

Materials & Methods

Adult C57Bl/6 mice were exposed to wideband noise (WBN; 91 or 96 dB SPL) for 6 hours/day for 3 days (***I***). We previously reported temporal threshold shifts with this protocol. Alternatively, mice were exposed to WBN at 110 dB SPL for 2 hours (***II***). Mice were then intra-peritoneally injected with fluorescently-conjugated gentamicin (GTTR, 2 mg/kg, in PBS, pH = 7.4). Thirty or sixty minutes later, paraformaldehyde (PFA)-fixed (4%) cochlear tissues were excised and processed for confocal microscopy and fluorescence quantification. Cardiac perfusion was omitted in a subset of mice to assess its effects.



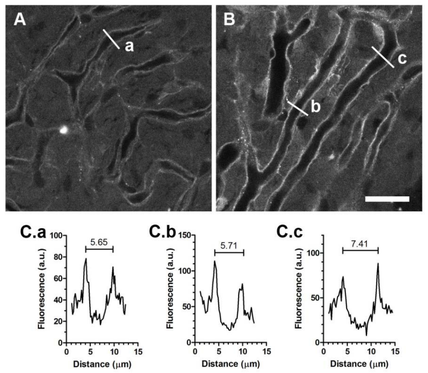
Intense GTTR fluorescence was typically observed in the endothelial cells of strial capillaries, allowing easy determination of capillary diameters using Fiji (NIH ImageJ) line profile function. The diameter was respectively defined either by the distance of intensity peaks, or by the width of an intensity plateau, depending on whether the lumen was identifiable or not. In practice, individual focal plane(s) representing strial capillary beds were first identified; then 60+ measures were acquired from apical, middle and basal cochlear locations. Appropriate t-tests was used to determine any significant differences between treatment groups. The examiner on diameter was blind to experimental conditions during analysis. All diameter measures were performed by one examiner except the dataset of 100 dB SPL, 2 hour sound treatment, in which an internal control without sound treatment was provided.

Results

1. Diameter measurement of strial capillaries, and sound-induced vasodilation

In order to determine the diameter of a segment of strial capillary, the appropriate focal plane for the segment from an image stack was first identified using ImageJ software (***A***, ***B***). The diameter of this segment was estimated by measuring the distance between the prominent peaks in the corresponding plot profile (*e.g.* ***C.a***) which reflects fluorescence intensity along a line (*e.g.* **a** in ***A***) perpendicular to the capillary axis. The scale bar is 20 µm.

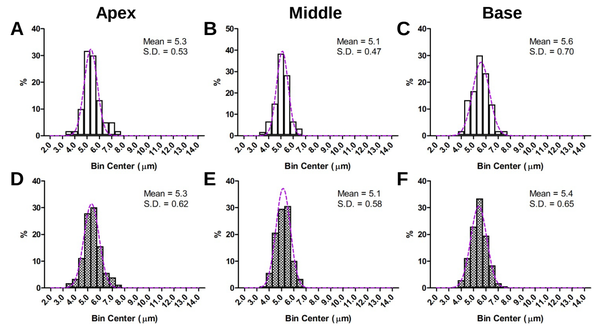
Sound treatment (WBN; 96 dB SPL, 6 hours/day for 3 days) enhanced fluorescence intensity in the intra-strial space in the stria vascularis (***B***) compared to the control tissue (***A***). In addition, sound treatment vasodilated strial capillaries, confirming the observation in our previous report [2].



2. Capillary diameters in control stria vascularis

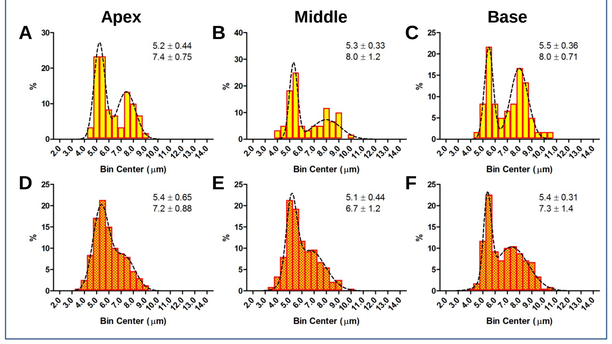
About 60 measures of capillary diameter were acquired from each image stack. The diameters from individual stack/site from individual control animals without sound treatment exhibited a Gaussian/normal distribution (***A-C***). The mean diameter varied between different cochlear locations. The value and standard deviation (S.D.) of the mean in µm are provided in each panel. Curve fitting in Gaussian distribution is depicted by a purple dotted line.

When measures were pooled from 3 animals from varied cochlear locations (180 total measures per location), the normal distribution upheld (***D-F***), and strial capillaries from the cochlear base present larger diameters than the cochlear apex (n.s., p=0.25, unpaired t test with Welch's correction), whilst strial capillaries from the mid-cochlear coils present significantly smaller diameters (p<0.0001, compared to base; p<0.0009, compared to apex; unpaired t test with Welch's correction).



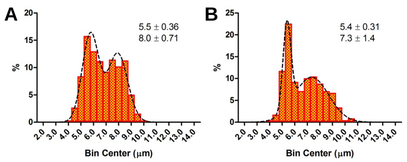
3. Capillary diameters in sound-treated strial vascularis

With sound treatment (96 dB SPL; WBN), a fraction of the strial capillaries retained their baseline diameter, while others dilated, forming a bi-modal distribution (***A-C***). Curve fitting in the sum of two Gaussians is depicted by a dotted line with both means (±S.D.) in µm displayed in each panel. Bi-modal distribution was not present in all surveyed sites, especially at more apical regions. Thus, when measures were pooled from 4 animals (240 total measures per location), the bi-modal distribution was less prominent (***D-F***) than the representative distribution from individual animals (***A-C***). Additionally, compared to control capillary diameters, sound-induced vasodilation was significant in the entire cochlea from apex to base (p<0.0001, Mann Whitney tests for apex, mid section or base).



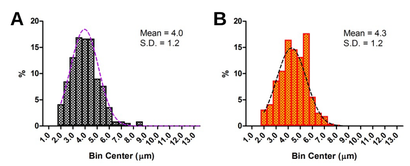
4. The effect of cardiac perfusion on sound-induced vasodilation in strial capillaries

To determine if cardiac perfusion during fixation with PFA affected capillary vasodilation, strial capillary diameters were measured in sound-treated mice (96 dB SPL; WBN) after cochlear excision and immersion fixation. Vasodilation of strial capillaries was evident from the basal region of sound-treated cochleae, showing a characteristic bi-modal distribution (***A***; 718 total measures). This distribution was not significantly different from strial capillary diameters from mice with cardiac perfusion fixation after 96 dB SPL, WBN sound exposure (***B***; p=0.29, Mann Whitney test).



5. The effect of shorter, more intense sound treatment on vasodilation

We also tested the effect of shorter, more intense sound treatment (WBN; 110 dB SPL, 2 hours) on strial vasodilation. Treatment by louder sound is more frequently found in literature of sound exposure. The diameter measurement was performed from the mid-base region of the cochlea. A Gaussian distribution was evident from the control tissue (***A***; n=3; 367 total measures). In sound-treated stria vascularis, capillaries were moderately dilated without a clear bi-modal distribution (***B***; n=4; 487 total measures). However, this vasodilation was significant (p<0.0001, unpaired t test with Welch's correction).



Discussion & Conclusions

Here we confirmed sound-induced capillary dilation in paraformaldehyde-fixed cochlear tissues. To our knowledge, the amount of dilation by chronic sound treatment surpassed previously-documented sound-induced capillary dilation *in vivo*, suggesting other pathological phenomena, e.g., inflammation, also occur. The bimodal distribution of dilation appears specific to sound conditions that enhanced cochlear uptake of GTTR, or elevated aminoglycoside uptake and exacerbated ototoxicity during endotoxemia [12].

In vivo measures of strial capillary diameter is challenging but achievable. Documented *in vivo* dilation is typically less than 10% of baseline diameter. For instance, inhalation of 5% CO_2 in oxygen induced 3.7% dilation in guinea pig strial capillaries [10]; and brief sound treatment at moderate level (85 dB SPL, 10 min) caused 7.5% dilation in mice [11]. The degree of these dilation measurements is comparable to our observations using short and intense sound treatment, but less than after prolonged loud sound treatment. The subtle dilation was likely an active physiological event in response to a need for higher cochlear blood flow. A 3.7% dilation was adequate for a 20% increase of blood flow [10]. On the contrary, the 30%+ dilation by prolonged sound treatment is likely a pathological event in the traumatized stria vascularis, facilitating drug trafficking, including aminoglycoside loading of the cochlea.

Angiotensive reagents or vasoconstrictors, including serotonin, endothelin-1 and angiotensin II, may counteract vasodilation and protect against ototoxicity by reducing aminoglycoside trafficking across the BLB. Similarly, vasodilatory agents and events, including loop diuretics, *Ginkgo biloba*, and inflammation may exacerbate aminoglycoside ototoxicity.

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Acknowledgements

Funded by NIH R03 DC011622 and W81XWH1410006 (HL), NIH R01 DC04555 (PSS) and P30 DC005983.

The authors would like to thank Dr. Meifang Xiao for her contributions to capillary diameter measurements.

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